UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

DATE: March 23, 2018

SUBJECT: **Zinc pyrithione:** Acticide® ZP 100M

PC Code: 088002	DP Barcode: 437459
Decision No.:	Registration Number(s): 67071-RNN
Petition No(s).: NA	Regulatory Action: Registration Review
Risk Assessment Type: NA	Case No.:
TXR No.: NA	CAS No.: 13463-41-7
MRID No.: 49788820	40 CFR: NA

FROM: Jorge G. Muñiz Ortiz, Toxicologist
Risk Assessment and Science Support Branch
Antimicrobials Division (7510P)

THROUGH: Laura Parsons, Acting Branch Chief
Tim Leighton, Senior Health Scientist
Risk Assessment and Science Support Branch
Antimicrobials Division (7510P)

TO: Aline Heffernan, Chemical Review Manager
Zeno Bain, Acting Team Lead
Regulatory Management Branch I (RMBI)
Antimicrobials Division (7510P)

The 90-day oral toxicity study combined with a neurotoxicity study in rats exposed to zinc pyrithione (MRID 49788820) was reviewed by the Agency and has been upgraded to acceptable.

EPA Reviewer: Jorge G. Muñiz Ortiz, Ph.D., DABT **Signature:**
RASSB, Antimicrobials Division (7510P) **Date:** March 22, 2018
EPA Secondary Reviewer: Tim McMahon, Ph.D. **Signature:**
RASSB, Antimicrobials Division (7510P) **Date:** March 22, 2018

TXR#: 1003401

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity study in rats (gavage) with 14-day recovery
OCSPP 870.3100; OECD 408

PC CODE: 088002 **DP BARCODE:** 437459

TEST MATERIAL (PURITY): ACTICIDE® ZP 100 (97.55% aqueous dispersion in 1% carboxymethyl cellulose)

SYNONYMS: zinc pyrithione

CITATION: A. Zmarowiski, Ph.D. (2014). 90-Day oral toxicity study combined with a neurotoxicity study with Zn Pyrithione by daily gavage in the rat followed by a 14-day recovery period. WIL Research Europe B.V., The Netherlands. Laboratory Project ID 501665. 15DEC2014. MRID 49788820 (unpublished).

SPONSOR: Thor Químicos de México, SA de CV

EXECUTIVE SUMMARY:

In a 90-day oral toxicity/neurotoxicity study (MRID 49788820), zinc pyrithione (Zn Pyr) 97.55% a.i.) prepared in 1% aqueous carboxymethyl cellulose was administered by gavage to groups of 5 or 10 (per sex) Wistar Han rats at doses of 0, 0.2, 0.5 and 2.5 mg/kg/day (See Table 1a). Additionally, there was a second main unexposed group (n = 5 per sex) and a second group exposed to 2.5 mg/kg (n = 5 per sex) used to study the potential effects of Zn Pyr 14 days after the 90-day exposure (See Table 1b). All of the animals in the groups selected as "Main" and "Recovery" were selected for functional observations and clinical laboratory investigations. All of the animals in the treated and control groups, except 5 each of each sex from the 0.2 mg/kg and 0.5 mg/kg treatment groups, were selected for perfusion at necropsy and histotechnology and histopathology. The reason for exclusion of these animals was not explained in the report.

Adverse effects from treatment were observed in both sexes at the 2.5 mg/kg dose, including hunched posture, (female rats only), lean appearance, abnormal gait, uncoordinated movements, hypotonia of the hind-legs, reduced landing foot splay at week 5, skeletal muscle atrophy, and swelling of the flank. In males, gait motility was significantly decreased at weeks 2-12 compared to pretest measurements. Positional passivity was also observed at a significantly higher percentage in females exposed to 2.5 mg/kg Zn Pyr when compared to unexposed females.

Decreased locomotor activity in males and females was also observed in rats exposed to 2.5 mg/kg Zn Pyr when compared to the unexposed group. In the male recovery group at 2.5 mg/kg/day, there was no difference in incidence of decreased locomotor activity between the unexposed and exposed recovery group. In females, the recovery group exposed to 2.5 mg/kg Zn Pyr showed a higher incidence of decreased locomotor activity than the unexposed group.

Males exposed to 2.5 mg/kg/day Zn Pyr also had lower brain width measurements than controls, but no other effects on the CNS were observed. In females exposed to 2.5 mg/kg Zn Pyr, significantly lower forelimb grip strength was observed at the end of treatment only. This effect was not observed in any other treatment group.

A statistically significant decrease in body weight was observed in female rats only exposed to 2.5 mg/kg where body weight gains were significantly lower from day 29 through the end of the treatment and through the end of the recovery period. This effect was statistically significant at the 0.05 confidence level at end of treatment and at the 0.01 confidence level at the end of recovery.

According to the hematology results, exposure to 2.5 and 0.5 mg/kg Zn Pyr showed a slight effect on the levels of white blood cells (WBC). In males exposed to 2.5 mg/kg and 0.5 mg/kg Zn Pyr the levels of WBC decreased by 28% and 32%, respectively. The levels of WBC were not statistically different during the recovery period between the controls and the rats exposed to 2.5 mg/kg. This effect was not observed in females or any other dose. Counts of hematocrit, red blood cells, as well as clinical biochemistry parameters such as chloride, inorganic phosphate, calcium, and creatinine were not considered to be toxicologically relevant as the values remained within the range considered normal for animals of this age and strain and/or were attributed to slightly high control values. Sodium levels in females exposed to 2.5 mg/kg Zn Pyr were significantly lower than the controls concomitantly with higher urine volumes. The lower levels of sodium might be due to the high volumes of urine diluting the sodium concentration. As noted above, the body weights of female rats exposed to 2.5 mg/kg of the chemical were also significantly lower than the controls. This suggests that exposure to 2.5 mg/kg of Zn Pyr induces the rats to lose body fluids via urine, which carries the sodium electrolyte, thus preventing body weight gain as compared to the controls.

After the 14-day recovery period, the animals showed a partial recovery. Hunched posture and abnormal gait were still evident for some females, though fore- and hind-limb grip strength normalized to control levels. Landing foot splay measurements did not reach control levels. The fat replacement in the microscopic examination had recovered at the end of the recovery period. The atrophy observed in skeletal muscle was present at a lower incidence and severity in both sexes at the end of recovery.

Increased ovary weight was observed in the recovery group exposed to 2.5 mg/kg when compared to the unexposed control group. The ovary weight differences were also observed in the recovery group by body weight percentage. The liver weight to body weight ratio was statistically higher in female rats exposed to 2.5 mg/kg than the rats in the unexposed control group. This effect persisted through the recovery period also at a statistically significant level. A similar effect in the recovery group of females exposed to 2.5 mg/kg compared to the unexposed controls was observed in the brain and the spleen.

Increased liver and spleen weights were observed at 2.5 mg/kg. However, there was an absence of histological findings, leading to the conclusion that these effects were not considered to be toxicologically relevant.

The macroscopic observations demonstrate that exposure to 2.5 mg/kg Zn Pyr can induce a reduction in size of the skeletal muscle only in female rats. The effect was observed in 3 out of 10 females in the exposed group; none of the rats in the unexposed group showed the effect. This effect is strongly correlated to the reduction in body weight in females exposed to 2.5 mg/kg Zn Pyr.

Males exposed to 0.5 mg/kg Zn Pyr demonstrated significantly ($p < 0.05$) higher grip strength in the fore-limb when compared to other exposure doses. The effect at the exposure dose of 0.5 mg/kg was observed at week 5 and at the end of recovery, but not during the pretest, or weeks 2 and 8. This effect was not significantly different between the unexposed controls and the other treatment groups.

A statistically significant ($p < 0.05$) increase in hindlimb grip strength was observed in female rats exposed to 2.5 mg/kg Zn Pyr at pretest. However, this effect began to be observed at lower doses during week 5 of exposure. A statistically significant ($p < 0.05$) effect observed in female rats exposed to 0.5 mg/kg, as opposed to male rats, was increased hind-limb grip strength. The effect was observed beginning at week 5 and continuing through week 8. This effect was not observed in female rats exposed at 0.2 mg/kg. Statistically significant ($p < 0.05$) decreases in hind- and forelimb grip strength were observed in female rats exposed to 2.5 mg/kg Zn Pyr at the end of treatment, compared to unexposed controls, as opposed to the higher grip strength observed at the 0.5 mg/kg dose. The effect observed at the end of treatment was not observed at the end of the recovery period.

Male rats exposed to 0.5 mg/kg Zn Pyr showed an increase in testes weight as well as an increase in seminal vesicle weight compared to unexposed controls. The seminal vesicle weight to body weight ratio was also higher in rats exposed to 0.5 mg/kg than the unexposed controls. The heart weight to body weight ratio was lower in rats exposed to 0.5 mg/kg compared to the unexposed controls. These effects were not observed at any other dose. Male rats exposed to 0.2 mg/kg Zn Pyr showed an increase in fixed brain mass when compared to the unexposed controls. Females exposed to 2.5 mg/kg showed an increase in ovary weight and ovary weight to body weight ratio compared to the unexposed controls at the end of the recovery period. Similar differences were observed in the brain, liver, and spleen weight to body weight ratio.

No effects of treatment were observed on arena observations, pupil reflex, rectal temperature, brain length and width measurements, and microscopic examination or ophthalmoscopy and food consumption at the 0.2 and 0.5 mg/kg Zn Pyr dose levels after 90 days of exposure.

In conclusion, following the oral administration of Zn Pyr for 90-days, toxicologically relevant effects characterized by clinical signs, lower body weights and relevant effects on the hindlimb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration were evident at 2.5 mg/kg. After a 14-day recovery period, these effects partially recovered.

The LOAEL from this study is established at 2.5 mg/kg, based on effects on the hindlimb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration. The NOAEL is established at 0.5 mg/kg.

This 90-day oral toxicity study in the rat is classified **acceptable/guideline.** Previously, this study had been classified as unacceptable due to missing data. However, the registrant submitted the information required to upgrade the study to acceptable.

In addition to the above, a recovery period of at least 28 days should be scheduled for follow-up according to the OCSPP 870.3100 guideline. In the submitted study, follow-up, or recovery was conducted for only 14 days. According to the conclusion, the rats partially recovered after the 14-day recovery period.

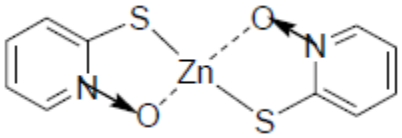
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: ***Zinc Pyrithione***

Description:	Whitish powder
Lot/batch #:	RP-270521-1608
Purity:	97.55% a.i.
Compound stability:	Stable
CAS # of TGAI:	*13463-41-7*
Structure:	



2. Vehicle and/or positive control: 1% aqueous carboxymethyl cellulose; water. Lot/Batch #; Not provided. Purity: Not provided.

3. Test animals:

Species: Rat
Strain: Crl:WI(Han) (outbred, SPF-Quality)
Age/weight at study initiation: ~6 weeks
Source: Charles River Deuthscland, Sulzfeld, Germany
Housing: 5 animals per sex
Diet: Free access to pelleted rodent diet *ad libitum*
Water: Free access to tap-water except during motor activity measurements *ad libitum*
Environmental conditions:

Temperature:	18-24°C
Humidity:	40-70%
Air changes:	10/hr
Photoperiod:	12 hrs dark/12 hrs light

Acclimation period: At least 5 days before the start of treatment under lab

conditions.

B. STUDY DESIGN:

1. **In life dates:** Start: 4DEC2013; End: 24MAR2014

2. **Animal assignment:** Animals were assigned by computer-generated random algorithm according to body weight, with all animals within ± 20% of the sex mean. Animals were assigned to the test groups as noted in Table 1.

TABLE 1a: Animals selected for functional observations and clinical laboratory investigations

Test group	Conc. in diet (N/A)	Dose to animal (mg/kg)	# Male	# Female
Control (Main)	Test material was given by gavage.	0	5	5
Control (Recovery)		0	5	5
Low		0.2	10	10
Mid		0.5	10	10
High (Main)		2.5	5	5
High (Recovery)		2.5	5	5

TABLE 1b: Animals selected for perfusion at necropsy and histotechnology and histopathology of perfused animals (these animals are a subset from Table 1a)

Test group	Conc. in diet (N/A)	Dose to animal (mg/kg)	# Male	# Female
Control (Main)	Test material was given by gavage.	0	5	5
Control (Recovery)		0	5	5
Low		0.2	5	5
Mid		0.5	5	5
High (Main)		2.5	5	5
High (Recovery)		2.5	5	5

3. **Dose selection rationale:** The dose levels for this study were selected based on the results from a 14-day range finding study (Project 503881) that demonstrated effects on food consumption at 3.4 mg/kg (350 ppm) in female beagle dogs. No effects on mortality, clinical signs, body weights, and ophthalmoscopic examination were observed. Based on that study the selected doses for the 90-day oral gavage study were 0, 0.2, 0.5 and 2.5 mg/kg.

4. **Test substance preparation:** Test substance was administered by oral gavage in this study at a rate of 2 ml/kg bw. Oral dosing solutions were prepared on a weight by weight basis in 1% aqueous carboxymethyl cellulose daily within 6 hours prior to dosing, and were homogenized to a visually acceptable level.

Results:

Homogeneity analysis: Homogeneity of dosing solutions was analyzed during Weeks 1, 6, and 13 on groups 2 and 4. The formulations of Group 2 and Group 4 were found homogenous (CoV ≤ 10%) for Week 1 to Week 13. Homogeneity of Group 3 formulations was established by interpolation. Samples were taken from the top (90% height), middle (50% height), or bottom (10% height) of the container. Preparation of formulations was considered acceptable if the

mean accuracy was in the range of 85-115% of the target concentration and if the coefficient of variation for homogeneity was ≤ 10%.

Stability analysis: Stability of the dose solutions was analyzed in week 1 from preparations to be given to Groups 2 and 4. Stability was measured at the time of exposure (t = 0 h and at t = 6 h) at room temperature and normal light conditions. Stability over the concentration range tested and over 6h at room temperature was confirmed in the dose range finding study (Project 503881).

Concentration analysis: Concentration analysis was performed on dosing solutions from groups 1-4. The mean analyzed concentrations of the Group 2 formulations were in the range of 83% and 94% of the target concentration. The mean accuracy for Group 2 formulations for Week 1 to Week 13 was 88%. The mean accuracies of the Group 3 formulations were between 82% and 92% of the target concentration. The mean accuracy of Group 3 formulations for Week 1 to Week 13 was 88%. The mean accuracies of the Group 4 formulations were between 90% and 102%. The mean accuracy of Group 4 formulations for Week 1 to Week 13 was 97%.

Concentration Analysis		
	Mean Analyzed Concentrations	Mean Accuracy (Week 1 - Week 13)
Group 1	No test substance detected	
Group 2	83%-94%	88%
Group 3	82%-92%	87%
Group 4	90%-102%	97%

(To see chemical analysis tables taken from the study report go to pages 18, 19, 20 of this document)

5. **Statistics:** Methods used:
   - If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex.
   - The Steel-test (many-to-one rank test) was applied if the data could not be assumed to follow a normal distribution.
   - The Fisher Exact-test was applied to frequency data.
   - The Kruskal-Wallis nonparametric ANOVA test was applied to motor activity data to determine intergroup differences.

All tests were two-sided and in all cases $p < 0.05$ was accepted as the lowest level of significance. Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables. Test statistics were calculated on the basis of exact values for means and pooled variances. Individual values, means and standard deviations may have been rounded off before printing. Therefore, two groups may display the same printed means for a given parameter, yet display different test statistics values.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected during pretest and again at weeks 2, 5, 8, 12 and 13 and at the end of the recovery period (0 mg/kg and 2.5 mg/kg treatments) for signs of toxicity and mortality.

1b. Clinical examinations: Clinical examinations were conducted at least once daily from the start of treatment onwards.

1c. Neurological evaluations: The following evaluations (measurements) were performed on Weeks 2, 5, 8, 12, and 13 and at the end of the recovery period on 5 main and 5 recovery period animals per sex of groups 1 and 4 and 10 main animals per sex in groups 2 and 3: signs of hearing ability, pupillary reflex, static righting reflex, fore- and hind-limb grip strength, landing hind-foot splay, locomotor activity, total movements and ambulations.

2. Body weight: Animals were weighed weekly.

3. Food consumption and compound intake: Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day.

4. Ophthalmoscopic examination: Eyes from all animals were examined at pretest and at Week 13, animals from Main Groups 1 (Control) and 4 (2.5 mg/kg dose) were examined.

5. Hematology and clinical chemistry: Blood was collected from Group 2 animals numbered 31-35 and 111-115 and Group 3 animals numbered 46-50 and 126-130 on 04MAR2014. Blood was collected from Group 1 (0 mg/kg) animals numbered 1-5, Group 2 (0.2 mg/kg) animals numbered 26-30, Group 3 (0.5 mg/kg) animals numbered 41-45, and Group 4 (2.5 mg/kg) animals numbered 56-60 on 06MAR2014. Blood was collected from Group 1 animals numbered 81-85, Group 2 animals numbered 106-110, Group 3 animals numbered 121-125, and Group 4 animals numbered 136-140 on 07MAR2014. Blood was collected from recovery Group 1 animals numbered 16-20 and 96-100 and Group 4 animals numbered 71-75 and 151-155 on 07MAR2014 and 21MAR2014. Samples were taken between 7:30 and 10:30 AM. Blood was collected for hematology and clinical chemistry from all surviving animals. Animals were fasted overnight (with a maximum of 24 h) but were provided with water. The CHECKED (X) parameters were examined.

a. Hematology:

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)*
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)*
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)*
x	Platelet count*	x	Reticulocyte count
	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
x	Calcium	x	Albumin*
x	Chloride	x	Creatinine*
	Magnesium	x	Urea nitrogen*
x	Phosphorus	x	Total Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	x	Total bilirubin
x	Alkaline phosphatase (ALK)*	x	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
x	Alanine aminotransferase (ALT/also SGPT)*		
x	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.310

6. **Urinalysis:** As part of the clinical laboratory investigations, urine was collected overnight from animals deprived of food but provided with water, which was the same dietary procedure performed for blood collection. The CHECKED (X) parameters were examined. Along with the parameters measured below, clarity, color, sodium, potassium, and calcium were also measured.

	Appearance*	x	Glucose
x	Volume*	x	Ketones
x	Specific gravity/osmolality*	x	Bilirubin
x	pH*	x	Blood/blood cells*
x	Sediment (microscopic)	x	Nitrate (samples were analyzed for nitrite)
x	Protein*	x	Urobilinogen

* Optional for 90-day oral rodent studies

7. **Sacrifice and pathology**: All rats that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM
x	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*+
	Gall bladder (not rat)*
	Bile duct (rat)
x	Pancreas*
X	**RESPIRATORY**
x	Trachea*
x	Lung*
x	Nose*
x	Pharynx*
x	Larynx*

X	CARDIOVASC./HEMAT.
x	Aorta*
xx	Heart*+
x	Bone marrow*
x	Lymph nodes*
xx	Spleen*+
xx	Thymus*+
X	**UROGENITAL**
xx	Kidneys*+
x	Urinary bladder*
xx	Testes*+
xx	Epididymides*+
xx	Prostate*
xx	Seminal vesicles*
xx	Ovaries*+
xx	Uterus*+
x	Mammary gland*

X	NEUROLOGIC
xx	Brain*+
x	Peripheral nerve*
x	Spinal cord (3 levels)*
x	Pituitary*
x	Eyes (optic nerve)*
X	**GLANDULAR**
xx	Adrenal gland*+
x	Lacrimal gland
x	Parathyroid*
xx	Thyroid*
X	**OTHER**
x	Bone (sternum and/or femur)
x	Skeletal muscle
x	Skin*
x	All gross lesions and masses*

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS:

The predominant effect observed from oral exposure to Zn Pyr was decreased motor activity at the 0.5 mg/kg and 2.5 mg/kg dose. Details on effects observed in this study are summarized below.

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Females at 2.5 mg/kg were observed with hunched posture, lean appearance, abnormal gait, uncoordinated movements, hypotonia of the hind-legs and swelling of the flank. Hunched posture and abnormal gait were seen for females at 2.5 mg/kg during the recovery period as well, though the abnormal gait normalized was no longer present during the second week of recovery.

2. **Mortality:** No mortality was observed during the study.

3. **Neurological evaluations:**
Arena observations: According to the results, no significant difference between exposed and unexposed male rats was observed for positional struggle or passivity, increased urination or feces production, hypersensitivity to touch, negative pinna reflex and loss of righting reflex at any dose level.

Decreased urination was observed in male rats at the 0.5 mg/kg dose at week 12 of the treatment period. This difference was not observed in the recovery groups; however the control percentage increased to the levels observed in the exposed groups. Decreased urination was also observed in unexposed females compared to those exposed to 0.5 mg/kg. There was no decrease in urination between female rats exposed to 2.5 mg/kg and unexposed

female rats. During the recovery period there was a significant difference in positional passivity between unexposed female rats compared to rats exposed to 2.5 mg/kg (35-45% vs. < 95%) and also in positional struggle (55-65% vs. 0%).

Pupil reflex: Pupil reflex was normal in treated and control animals at all dose levels throughout the treatment.

Foot splay: Effects of Zn Pyr on landing foot splay were observed at 2.5 mg/kg. Males had reduced foot splay measurements vs. controls during weeks 5, 8, 12 (end of treatment) and persisted throughout the recovery period. The effect began to be observed on week 2, where the distance in males treated at 2.5 mg/kg Zn Pyr was 46.3 mm ± 11.3 vs. 59.4 mm ± 14.0 in unexposed males ($p < 0.05$). At week 5 the effect was stastistically significant at $p < 0.01$ (52.1 mm ± 15.9 vs. 75.3 mm ± 13.7). The difference in foot splay was also observed on week 8 (50.8 mm ± 12.4 vs. 71.9 mm ± 8.8) and at the end of treatment (51.4 mm ± 12.1 vs. 73.6 mm ± 7.6; $p < 0.01$) in males exposed to 2.5 mg/kg compared to unexposed males. This effect was observed during the recovery time also at a $p < 0.05$ (71.6 mm ± 7.2 vs. 87.8 mm ± 11.8). The difference between treatments compared to unexposed males was not observed at any other dose. Females had smaller foot splay measurements only at week 5 (exposed 50.1 mm ± 7.1 vs. unexposed 61.8 mm ± 15.9; $p < 0.05$), though this was considered relevant based on other clinical signs and microscopic findings.

Grip strength: Females exposed to 2.5 mg/kg Zn Pyr showed significantly lower fore- (774.8 g ± 74.8 vs. 870.2 g ± 108.4; $p < 0.05$) and hindlimb (596.1 g ± 62.2 vs. 683.3 g ± 56.5; $p < 0.05$) grip strength vs. unexposed controls at the end of the treatment period. Hind limb grip strength was higher in female rats exposed to 0.5 mg/kg Zn Pyr compared to unexposed rats at week 5 (519.8 g ± 46.5 vs. 449.7 g ± 62.1; $p < 0.05$) and at week 8 (627.1 g ± 125.5 vs. 521.6 g ± 69.4; $p < 0.05$). The differences in grip strength were not evident by the end of the treatment and recovery periods. Males exposed to 0.5 mg/kg Zn Pyr showed an increase on forelimb grip strength compared to unexposed males (655.2 g ± 112.8 vs. 524.2 g ± 87.7; $p < 0.05$) on week 5 and at the end of treatment (1056.5 g ± 137 vs. 865.2 g ± 865.2 ± 185.9; $p < 0.05$). This effect was not observed at the end of treatment.

Motor activity: Even though the petitioners state that there were no toxicologically relevant differences in motor activity between the treated and untreated rats, the results show that there was a dose-dependent decrease in locomotor activity in males and females during the treatment period. There was a dose-response relationship in decreased locomotor activity from exposure to Zn Pyr. At the end of treatment 55-65% of unexposed males had decreased locomotor activity compared to > 95% of males exposed to 2.5 mg/kg Zn Pyr. These differences observed during the treatment period were not observed at the end of the recovery period in males, however, the difference was still observed in female rats at end of the recovery period (0% vs. 15-25%).

B. BODY WEIGHT AND WEIGHT GAIN:

The most significant body weight changes (weight decrease) were observed in females exposed to 2.5 mg/kg /day Zn Pyr from day 36 onwards. Weight was 16% higher in unexposed females at the end of treatment and 18% higher at end of recovery (see graph below).

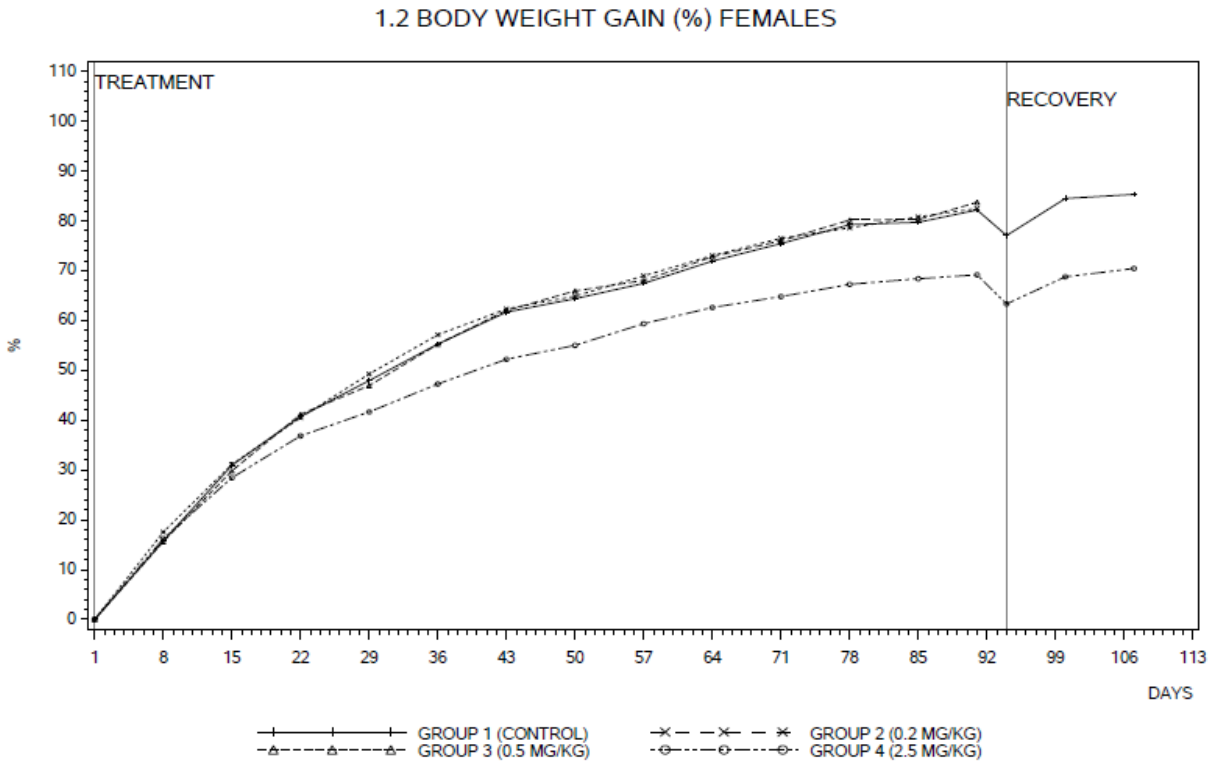


TABLE 2. Average body weights and body weight gains during 90 days of treatment [a]						
Dose mg/kg	**Body weights (g ± SD)**				**Total weight gain**	
	Pre Test	**Week 1**	**Week 7**	**Week 13**	**g**	**% of control**
Male						
0	No Pre Test Body Weight data was provided	159 ± 7.5	350 ± 32.6	421 ± 39.0	262	164 ± 20.1
0.2		162 ± 5.9	354 ± 20.2	427 ± 26.4	265	164 ± 14.8
0.5		158 ± 8.3	341 ± 22.1	441 ± 30.9	283	160 ± 11.6
2.5		160 ± 7.6	341 ± 26.5	403 ± 31.6	243	151 ± 16.7*
Female						
0	No Pre Test Body Weight data was provided	133 ± 6.9	215 ± 12.6	242 ± 15.8	109	82 ± 13.7
0.2		128 ± 5.0	207 ± 10.0	234 ± 11.4	106	82 ± 10.2
0.5		128 ± 9.3	207 ± 10.4	235 ± 14.1	107	84 ± 11.5
2.5		132 ± 7.2	200 ± 17.3**	223 ± 20.6**	91	69 ± 14.3**

[a] Data obtained from pages 69-74 of 550 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

No pre-test body weight data were provided in the study report. This is considered a deficiency, and the registrant should provide these data for this study.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. Food consumption: There were no significant differences in reported food consumption among and between any of the treatment groups compared to unexposed controls at any time point throughout the experiment.

D. OPHTHALMOSCOPIC EXAMINATION: There were no ophthalmology findings at the pre-dose or at the end of treatment.

E. BLOOD ANALYSES:

1.15 HAEMATOLOGY SUMMARY
MALES

		GROUP 1 CONTROL	GROUP 2 0.2 MG/KG	GROUP 3 0.5 MG/KG	GROUP 4 2.5 MG/KG
END OF TREATMENT					
WBC	MEAN	7.8	6.3	5.3 **	5.6 *
10E9/L	ST.DEV	2.0	1.5	1.4	1.5
	N	10	10	10	10
Neutrophils	MEAN	19.0	18.7	18.1	21.0
%WBC	ST.DEV	4.2	4.1	3.3	5.4
	N	10	10	10	10
Lymphocytes	MEAN	77.0	77.8	78.5	74.9
%WBC	ST.DEV	4.8	4.5	3.4	5.7
	N	10	10	10	10
Monocytes	MEAN	2.2	1.9	1.9	2.1
%WBC	ST.DEV	0.6	0.3	0.5	0.6
	N	10	10	10	10
Eosinophils	MEAN	1.6	1.4	1.4	1.8
%WBC	ST.DEV	0.6	0.5	0.4	0.5
	N	10	10	10	10
Basophils	MEAN	0.2	0.2	0.1	0.2
%WBC	ST.DEV	0.1	0.1	0.1	0.1
	N	10	10	10	10
Red blood cells	MEAN	9.11	8.85	8.92	8.65 *
10E12/L	ST.DEV	0.36	0.35	0.36	0.38
	N	10	10	10	10

+/++ Steel-test significant at 5% (+) or 1% (++) level
*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

1.15 HAEMATOLOGY SUMMARY
MALES

		GROUP 1 CONTROL	GROUP 2 0.2 MG/KG	GROUP 3 0.5 MG/KG	GROUP 4 2.5 MG/KG
END OF RECOVERY					
MCH	MEAN	1.10			1.15 *
fmol	ST.DEV	0.03			0.02
	N	4			5

+/++ Steel-test significant at 5% (+) or 1% (++) level
*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

1.15 HAEMATOLOGY SUMMARY
FEMALES

		GROUP 1 CONTROL	GROUP 2 0.2 MG/KG	GROUP 3 0.5 MG/KG	GROUP 4 2.5 MG/KG
END OF TREATMENT					
Haematocrit	MEAN	0.450	0.453	0.444	0.432 **
L/L	ST.DEV	0.012	0.010	0.013	0.012
	N	10	10	10	10

+/++ Steel-test significant at 5% (+) or 1% (++) level
*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

1. **Hematology:** According to the hematology results, exposure to 2.5 and 0.5 mg/kg Zn Pyr showed a slight effect on the levels of white blood cells (WBC). In males exposed to 2.5 mg/kg and 0.5 mg/kg Zn Pyr the levels of WBC decreased by 28% and 32%, respectively (5.6×10^9/L ± 1.5 vs. 7.8×10^9/L ± 2.0; $p < 0.05$ and 5.3×10^9/L ± 1.4 vs. 7.8×10^9/L ± 2.0; $p < 0.01$, respectively). The levels of WBC were not statistically different during the recovery period between the controls and the male and female rats exposed to 2.5 mg/kg. This effect was not observed in females or any other dose. Even though there were statistically significant differences in hematocrit between female rats exposed to 2.5 mg/kg Zn Pyr compared to unexposed rats, the difference is not considered toxicologically relevant.

2. **Clinical chemistry:** Chloride was significantly increased (107 ± 1 mmol/L vs. 105 ± 2 mmol/L; p ¸0.05) and inorganic phosphate (1.73 ± 0.13 mmol/L vs. 1.92 ± 0.19 mmol/L; $p < 0.05$) and calcium (2.44 ± 0.08 mmol/L vs. 2.54 ± 0.05 mmol/L; $p < 0.01$) were significantly lower when measured at the end of the treatment in males exposed to 2.5 mg/kg Zn Pyr. However, these effects are not toxicologically relevant. Calcium was significantly lower for males exposed to 0.5 mg/kg at the end of treatment (2.46 ± 0.06 mmol/L vs. 2.54 ± 0.05 mmol/L; $p < 0.05$) and significantly lower when exposed to 2.5 mg/kg Zn Pyr measured at the end of the recovery period (2.52 ± 0.04 mmol/L vs. 2.60 ± 0.02 mmol/L; $p < 0.01$). However, these effects are not toxicologically relevant. Creatinine was significantly lower for females exposed to 2.5 mg/kg both at the end of treatment (37. ± 3.9 0 µmol/L vs. 43 ± 2.1 µmol/L; $p < 0.01$) and recovery periods (40.8 ± 2.8 µmol/L vs. 44.5 ± 1.8 µmol/L; $p < 0.05$). These effects are not toxicologically relevant.

F. URINALYSIS:

There was a statistically significant ($p < 0.01$) difference in the volume of urine in female rats exposed to 2.5 mg/kg Zn Pyr compared to the unexposed controls. The urine volume of exposed females at the highest concentration was 17 mL ± 9 and the volume in unexposed females was 10 mL ± 4. The specific gravity was also significantly lower in the exposed rats when compared to the unexposed rats (1.014 ± 0.006 mL vs. 1.022 ± 0.006 mL; $p < 0.05$). In addition, the levels of the sodium electrolyte in the urine were significantly lower in female rats exposed to 2.5 mg/kg Zn Pyr when compared to the unexposed controls (28.5 ± 10.8 mmol/L vs. 54.9 ± 13.8 mmol/L; $p < 0.01$). The lower levels of sodium in the urine could be due to the high levels of urine in the exposed rats, which would be diluting the concentration of sodium. The higher volume, lower specific gravity and lower sodium levels in the urine could imply an effect on concentrating of the urine by the kidneys. However, damage to the kidneys from histopathology examination were not observed.

G. SACRIFICE AND PATHOLOGY:

The tables below represent only the incidence gradings from the microscopic results of the effects observed from exposure of male and female rats to Zn Pyr in the skeletal muscle, fatty tissues and axons (Data taken from pages 305-307 of the study report). The results were from observations made in the main exposure groups and the recovery exposure groups.

EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

M	= Male animal
F	= Female animal
K0	= Terminal sacrifice group
R1...R9	= Recovery / post-treatment group 1...9

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G	= Gross observation checked off histologically
*	= Comment in text of individual animal data
'	= Histologic examination not required
+	= Organ examined, findings present
-	= Organ examined, no pathologic findings noted (AOFT only)
(	= Only one of paired organs examined/present

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1	= Minimal / very few / very small
GRADE 2	= Slight / few / small
GRADE 3	= Moderate / moderate number / moderate size
P	= Finding present, severity not scored
(	= Finding unilateral in paired organs
*	= Comment in text of individual animal data

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0)
Incidence table - Selected findings with grades

Sex		Males				Females			
Dose Group		01	02	03	04	01	02	03	04
No. Animals per Dose Group		10	10	10	10	10	10	10	10
SKELETAL MUSCLE	No.Examined	10	10	10	10	10	10	10	10
- Atrophy	GRADE 1	-	-	-	1	-	-	-	1
	GRADE 2	-	-	-	1	-	-	-	2
	GRADE 3	-	-	-	-	-	-	-	3
	TOTAL AFFECTED	-	-	-	2	-	-	-	6
	MEAN GRADE/TISS.AFFECTED	-	-	-	1.5	-	-	-	2.3
- Fat replacement	GRADE 1	-	-	-	-	-	-	-	1
	GRADE 2	-	-	-	-	-	-	-	2
	TOTAL AFFECTED	-	-	-	-	-	-	-	3
	MEAN GRADE/TISS.AFFECTED	-	-	-	-	-	-	-	1.7
- Axonal degeneration	GRADE 1	-	-	-	-	-	-	-	1
	GRADE 2	-	-	-	-	-	-	-	1
	TOTAL AFFECTED	-	-	-	-	-	-	-	2
	MEAN GRADE/TISS.AFFECTED	-	-	-	-	-	-	-	1.5

Group 01, CONTROL, males: Zinc pyrithione (0 MG/KG); females: Zinc pyrithione (0 MG/KG)
Group 02, 0.2 MG/KG, males: Zinc pyrithione (0.2 MG/KG); females: Zinc pyrithione (0.2 MG/KG)
Group 03, 0.5 MG/KG, males: Zinc pyrithione (0.5 MG/KG); females: Zinc pyrithione (0.5 MG/KG)
Group 04, 2.5 MG/KG, males: Zinc pyrithione (2.5 MG/KG); females: Zinc pyrithione (2.5 MG/KG)

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
Necropsy Status: RECOVERY / POST-TREATMENT GROUP (R1)
Incidence table - Selected findings with grades

Sex		Males				Females			
Dose Group		01	02	03	04	01	02	03	04
No. Animals per Dose Group		5	-	-	5	5	-	-	5
SKELETAL MUSCLE	No.Examined	5	-	-	5	5	-	-	5
- Atrophy	GRADE 1	-	-	-	1	-	-	-	1
	GRADE 2	-	-	-	-	-	-	-	1
	TOTAL AFFECTED	-	-	-	1	-	-	-	2
	MEAN GRADE/TISS.AFFECTED	-	-	-	1.0	-	-	-	1.5

Group 01, CONTROL, males: Zinc pyrithione (0 MG/KG); females: Zinc pyrithione (0 MG/KG)
Group 02, 0.2 MG/KG, males: Zinc pyrithione (0.2 MG/KG); females: Zinc pyrithione (0.2 MG/KG)
Group 03, 0.5 MG/KG, males: Zinc pyrithione (0.5 MG/KG); females: Zinc pyrithione (0.5 MG/KG)
Group 04, 2.5 MG/KG, males: Zinc pyrithione (2.5 MG/KG); females: Zinc pyrithione (2.5 MG/KG)

1. **<u>Organ weight</u>:**

Male rats exposed to 0.5 mg/kg zinc pyrithione showed a statistically significant ($p < 0.05$) increase in testes weight (3.55 ± 0.26 g vs. 3.86 ± 0.25 g) as well as a statistically significant increase ($p < 0.05$) in seminal vesicle weight compared to unexposed controls (1.367 ± 0.176 g vs. 1.675 ± 0.193 g). The seminal vesicle weight to body weight ratio was also higher ($p < 0.05$) in rats exposed to 0.5 mg/kg than the unexposed controls (0.341% ± 0.046 vs. 0.426% ± 0.050). The heart weight to body weight ratio was significantly ($p < 0.05$) lower in rats exposed to 0.5 mg/kg compared to the unexposed controls (0.271% ± 0.013 vs. 0.255% ± 0.011). These effects were not observed at any other dose. Male rats exposed to 0.2 mg/kg zinc pyrithione showed a statistically significant ($p < 0.01$) increase in fixed brain mass when compared to the unexposed controls (2.261 ± 0.1 g vs. 2.487 ± 0.105 g).

Females exposed to 2.5 mg/kg showed an increase in liver weight to body weight ratio (2.70 ± 0.22 g vs. 2.47 ± 0.21 g; $p < 0.05$) at the end of recovery compared to unexposed female rats. Females exposed to that same dose showed an increase in ovary weight (0.154 ± 0.017 g vs. 0.125 ± 0.011 g; $p < 0.05$) and ovary weight to body weight ratio (0.071 ± 0.010 g vs. 0.053 ± 0.004 g; $p < 0.01$) compared to the unexposed controls at the end of the recovery

period. Similar differences were observed in the brain (0.87 ± 0.02 g vs. 0.82 ± 0.04 g; $p < 0.05$), liver (2.44 ± 0.13 g vs. 2.27 ± 0.09 g; $p < 0.05$), and spleen (0.215 ± 0.019 g vs. 0.181 ± 0.025 g; $p < 0.05$) weight to body weight ratio.

2. Gross pathology:

At 2.5 mg/kg, visibly reduced size of the skeletal muscle was noted for 3 out of 10 females and 2 out of 10 males. These signs were attributable to treatment and were considered adverse. Emaciation and reduction in size of the skeletal muscle was observed in the same 2 out of 10 female rats exposed to 2.5 mg/kg Zn Pyr. Incidental macroscopic findings seen are not considered to be toxicologically relevant.

According to the petitioner incidental findings included exophthalmus or enlarged eye (no difference in incidence from unexposed males vs. exposed males at 2.5 mg/kg dose), pelvic dilation of the kidneys, enlarged thyroid, reddish or tan focus/foci on the stomach glandular mucosa, clitoral gland, mesenteric lymph node or thymus, discoloration of the thymus, uterus contains fluid, diaphragmatic hernia, reduced size of the thyroid gland, and alopecia (15-25% of males exposed to 2.5 mg/kg Zn Pyr vs. 0% of unexposed males at end of treatment or end of recovery. Unexposed females had a higher incidence, 5-15% at end of treatment and 15-25% at end of recovery than any exposure group). They state that these effects are commonly seen for animals of this age and strain and did not show a dose related trend. Therefore, these effects were not considered toxicologically relevant.

3. Microscopic pathology:

Treatment related microscopic findings of skeletal muscle were present in the main and recovery 2.5 mg/kg treated animals. Atrophy was observed in 2 of out of 10 main group male and 6 out of 10 females up to a moderate degree. This effect was also observed in 1 out of 5 males and 2 out of 5 females after the end of recovery after being exposed to 2.5 mg/kg Zn Pyr. This effect was correlated to the macroscopic reduction in body weight of the treated animals compared to the non-exposed controls.

Fat replacement of the skeletal muscle was present in 3 out of 10 main females up to a slight degree after exposure to 2.5 mg/kg Zn Pyr.

Axonal degeneration was present up to a slight degree in the nerve branches in the affected skeletal muscles of 2 out of 10 females exposed to 2.5 mg/kg Zn Pyr.

After the 14-day recovery period, the fat replacement and axonal degeneration of the skeletal muscle had recovered (was not present).

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Exposure to 2.5 mg/kg of Zn Pyr for 90 days results in clinical signs, lower body weights/weight gains and relevant effects on the hind-limb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration, which are of toxicological relevance. A NOAEL of 0.5 mg/kg was established by the authors from the results of this study.

B. REVIEWER COMMENTS:

No discrepancies between what was observed, the conclusions to which the investigators came to and this reviewer were noticed. According to the results of this study the LOAEL was established at 2.5 mg/kg based on effects on the hindlimb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration. A NOAEL of 0.5 mg/kg was established based on the results presented in the report. The reviewer agrees with the LOAEL established by the petitioner.

The data analyzed and the conclusions reached by the reviewer cannot be confirmed at this time because some of the rats were not accounted for in the study. Therefore, this 90-day oral toxicity study in the rat is classified **acceptable/guideline**. Previously, this study had been classified as unacceptable due to missing data. However, the registrant submitted the information required to upgrade the study to acceptable.

C. STUDY DEFICIENCIES:

According to the OCSPP 870.3100 guideline, a recovery period of at least 28 days should be scheduled for follow-up. In the submitted study, follow-up, or recovery was conducted for only 14 days. According to the conclusion, the rats partially recovered after the 14-day recovery period. A 28-day recovery period may have resulted in more complete recovery.

Accuracy and homogeneity test – Week 1

Table 2 Accuracy and homogeneity test - Week 1

Group	Date of analysis	Sample position	Concentration [mg/g]		Accuracy [%]		Homogeneity (coefficient of variation) [%]
			Target	Analysed	Individual	Mean	
1	04-Dec-2013	50% height	0.00	n.d.	n.a.	n.a.	n.a.
			0.00	n.d.	n.a.		
2	04-Dec-2013	90% height	0.100	0.0981	98	94	4.7
			0.100	0.0905	90		
		50% height	0.100	0.0913	91		
			0.100	0.0933	93		
		10% height	0.100	0.101	101		
			0.100	0.0897	90		
3	04-Dec-2013	50% height	0.250	0.229	92	92	n.a.
			0.250	0.232	93		
4	04-Dec-2013	90% height	1.25	1.27	101	102	3.7
			1.25	1.19	95		
		50% height	1.25	1.29	103		
			1.25	1.33	106		
		10% height	1.25	1.26	101		
			1.25	1.29	103		

n.d. Not detected.
n.a. Not applicable.

Table 3 Accuracy and homogeneity test - Week 6 and extra analysis of Group 2 in Week 7

Group	Date of analysis	Sample position	Concentration [mg/g]		Accuracy [%]		Homogeneity (coefficient of variation) [%]
			Target	Analysed	Individual	Mean	
1	08-Jan-2014	50% height	0.00	n.d.	n.a.	n.a.	n.a.
			0.00	n.d.	n.a.		
2	08-Jan-2014	90% height	0.100	0.0882	88	83	8.4
			0.100	0.0882	88		
		50% height	0.100	0.0726	73		
			0.100	0.0779	78		
		10% height	0.100	0.0904	90		
			0.100	0.0810	81		
2#	15-Jan-2014	90% height	0.100	0.0948	95	91	6.3
			0.100	0.0825	82		
		50% height	0.100	0.0889	89		
			0.100	0.0925	93		
		10% height	0.100	0.0994	99		
			0.100	0.0898	90		
3	08-Jan-2014	50% height	0.250	0.227	91	88	n.a.
			0.250	0.210	84		
4	08-Jan-2014	90% height	1.25	1.13	90	90	2.0
			1.25	1.10	88		
		50% height	1.25	1.11	89		
			1.25	1.15	92		
		10% height	1.25	1.12	90		
			1.25	1.16	93		

Extra analysis of Group 2 in Week 7
n.d. Not detected.
n.a. Not applicable.

Table 4 Accuracy and homogeneity test - Week 13

Group	Date of analysis	Sample position	Concentration [mg/g]		Accuracy [%]		Homogeneity (coefficient of variation) [%]
			Target	Analysed	Individual	Mean	
1	26-Feb-2014	50% height	0.00	n.d.	n.a.	n.a.	n.a.
			0.00	n.d.	n.a.		
2	26-Feb-2014	90% height	0.100	0.0831	83	83	2.7
			0.100	0.0866	87		
		50% height	0.100	0.0840	84		
			0.100	0.0841	84		
		10% height	0.100	0.0797	80		
			0.100	0.0832	83		
3	26-Feb-2014	50% height	0.250	0.206	82	82	n.a.
			0.250	0.203	81		
4	26-Feb-2014	90% height	1.25	1.24	100	99	5.3
			1.25	1.25	100		
		50% height	1.25	1.12	90		
			1.25	1.30	104		
		10% height	1.25	1.21	97		
			1.25	1.29	103		

n.d. Not detected.
n.a. Not applicable.